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Research Article

Community DNA outperforms eDNA metabarcoding for biodiversity assessments in the Clarion–Clipperton Fracture Zone

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Abstract

Metabarcoding offers a powerful approach for assessing benthic biodiversity in remote and understudied deep-sea environments. However, the methodological performance of different DNA extraction strategies remains insufficiently evaluated, especially for benthic meiofaunal communities. In this study, we compared two extraction strategies—community DNA (ComDNA) from isolated benthic metazoans and sedimentary environmental DNA (SedDNA) from whole sediment—to assess benthic metazoan diversity in the Clarion-Clipperton Fracture Zone (CCZ), an area targeted for future polymetallic nodule mining. Using the 18S V1–V2 rRNA marker, we analyzed alpha and beta diversity, taxonomic composition, and the number of metazoan operational taxonomic units (OTUs) recovered by each strategy. ComDNA extractions yielded substantially higher benthic metazoan OTU richness (2,145 OTUs) than SedDNA (392 OTUs), with only 1.2% of OTUs shared between them. Community composition also differed significantly, driven by strategy-specific detection biases. To evaluate the effectiveness of SedDNA for biodiversity monitoring, we modeled the sediment volume required to recover OTU richness comparable to ComDNA samples. Depending on sequencing depth and statistical approach, we estimated that the processing of 27–82 mL of sediment is necessary to match ComDNA-derived richness. Our findings underscore the superior taxonomic resolution of ComDNA extractions but also highlight the potential of optimized SedDNA protocols for scalable biodiversity monitoring. We recommend sediment homogenization, increased sample volume, and a higher sequencing depth of at least 100,000 reads per sample for improving SedDNA-based assessments, particularly in heterogeneous deep-sea environments. These results provide critical methodological guidance for the development of standardized, efficient monitoring strategies in the context of deep-sea mining impact assessments.

Key words: Benthic metazoan, Clarion-Clipperton Fracture Zone (CCZ), deep-sea, DNA extraction, environmental DNA, metabarcoding, polymetallic nodules

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Introduction

The largely unexplored and remote expanses of the deep-sea floor form the largest ecosystem on Earth, covering more than 60% of the planet's surface (Dell'Anno et al. 2015; Tyler et al. 2016). The flattest parts of the deep-sea floor, the abyssal plains (Hernández-Molina et al. 2008), represent the world's largest benthic environment (Tyler et al. 2016). Habitat heterogeneity, at both local and regional scales, within the vast expanse of these abyssal plains is one of the factors promoting high biodiversity (Durden et al. 2015; Tyler et al. 2016; Paulus 2021). Within these benthic ecosystems, meiofaunal organisms are the most abundant metazoans (Sinniger et al. 2016; Pape et al. 2017), thriving in high densities within the upper 5 cm of the sediments (Lins et al. 2021; Lefaible et al. 2023; Uhlenkott et al. 2021; Uhlenkott et al. 2023). Meiofauna refers to the size class of animals that pass through a 1 mm sieve but are retained on a 32 µm sieve (Giere 2009; Lins et al. 2021; Uhlenkott et al. 2021). These organisms are key contributors to benthic ecosystem functions, including nutrient cycling and decomposition. They influence geochemical cycles of carbon and nitrogen, affect hydrodynamics through sediment reworking, and shape the composition and activity of microbial communities (Snelgrove et al. 1997; Schratzberger and Ingels 2018; Gielings et al. 2021). Meiofauna also serve as a vital link in benthic food webs, connecting decomposed organic matter with higher trophic levels (Montagna 1984; Coull 1999; Schratzberger and Ingels 2018).

Despite their vastness and remoteness, deep-sea environments are increasingly threatened by anthropogenic activities such as fossil fuel extraction (Glover and Smith 2003), marine litter (Kane and Clare 2019), and the cascading effects of climate change (Sweetman et al. 2017). Another emerging pressure is deep-sea mineral mining (Hein et al. 2020), driven by rising global demand for metals and mineral resources (Koljonen et al. 2024).

Marine mineral deposits include massive sulfides that form at hydrothermal venting sites, cobalt-rich crusts on seamount flanks (Koschinsky et al. 2018; Petersen et al. 2020), and polymetallic nodules that are found at abyssal plains worldwide. They form through precipitation of metals from sediment porewater and seawater and are of particular interest due to their high concentrations of manganese, copper, nickel, and cobalt (Kuhn et al. 2017). Extensive nodule fields occur in the Clarion–Clipperton Fracture Zone (CCZ), which contains an estimated 21 billion tons of nodules (ISA 2010) and is therefore a major target area for potential mining operations (Hein and Koschinsky 2014; Kuhn et al. 2017).

Although only prototype mining vehicles have been tested so far, several collector concepts are under active development, ranging from tracked vehicles connected to surface vessels via riser pipes to emerging autonomous underwater vehicle (AUV)-based systems (van Nijen et al. 2018; Hein et al. 2020; Setså 2023; Lefaible et al. 2023). Depending on the technology used, nodule extraction is expected to have moderate to severe impacts on benthic ecosystems (Lins et al. 2021; Li et al. 2022). Nevertheless, mining will create both direct impacts, caused by the removal of nodules and surficial sediments, and indirect impacts, including sediment plumes generated by the collector, as well as noise and light pollution (Shulse et al. 2017; Boetius and Haeckel 2018; Lins et al. 2021). The sediment plume, which can spread for several kilometers away from the collector, will affect the near-bottom waters due to higher particle

concentrations and the seafloor through redeposition and blanketing (Smith et al. 2010; Lefaible et al. 2023; Gazis et al. 2025). It will disperse organisms and bury benthic fauna due to the settling of the generated plume (Lins et al. 2021). Recent studies highlight the potential detrimental effects of seafloor disturbance on deep-sea biodiversity, revealing light to substantial losses of benthic fauna in disturbed areas (Stratmann et al. 2021; Lefaible et al. 2023) with persistent physical footprints even after four decades (Jones et al. 2025).

Maintaining meiofaunal and broader benthic biodiversity is essential, particularly as a substantial part of faunal diversity is still unknown (Sinniger et al. 2016), and numerous species found in the deep sea, especially in the CCZ, still need to be described (Smith et al. 2008; Lejzerowicz et al. 2021).

Monitoring deep-sea ecosystems

Considering the effects of climate change and increasing anthropogenic pressures on marine environments, robust monitoring programs are essential for assessing ecosystem health and quality (Zeppilli et al. 2015; Lins et al. 2021). Equally critical are environmental impact assessments (EIA), which evaluate how expanding human activities affect pristine, already disturbed, or even threatened marine ecosystems (Sinniger et al. 2016; Lefaible et al. 2023). Insights from these efforts underpin both biodiversity conservation and the maintenance of ecosystem functions and services (Lins et al. 2021), a need that is particularly acute in the context of polymetallic nodule mining (Wedding et al. 2015; Miller et al. 2018).

Traditional monitoring of benthic ecosystems primarily relies on the morphological identification of meio- and macroinvertebrates (Hestetun et al. 2021). This approach involves extracting the targeted organisms from sediment samples for subsequent identification and quantification. However, the process of extracting, sorting, and identifying benthic organisms, particularly meiofauna, is highly time-consuming and requires specialized taxonomic expertise, which is both rare and costly (Brannock and Halanych 2015; Lejzerowicz et al. 2015; Rzeznik-Orignac et al. 2017; Hestetun et al. 2021; Lins et al. 2021; Pawlowski et al. 2022). In addition, identifying meiofaunal organisms to species level based on morphological characters is particularly challenging due to their small size, overlapping morphological traits (Dell'Anno et al. 2015; Duarte et al. 2021), and the risk of feature damage during the extraction process (Castro et al. 2021). Furthermore, many species in deep-sea benthic habitats remain undescribed (Glover et al. 2018; Lejzerowicz et al. 2021).

Given these challenges, molecular methods provide a promising alternative for cost-effective and rapid biodiversity assessment in marine benthic ecosystems, particularly for evaluating anthropogenic impacts (Derycke et al. 2021).

Molecular methods such as environmental DNA (eDNA) metabarcoding (Taberlet et al. 2012c) provide a powerful and rapid tool for monitoring benthic ecosystems, quantifying biodiversity, and assessing anthropogenic impacts (Sinniger et al. 2016; Brandt et al. 2020). DNA can be obtained either from organisms that have been separated from their substrate (e.g., sediment) prior to extraction, referred to as community DNA (ComDNA) (Creer et al. 2016; Deiner et al. 2017), or directly from the sediment, which is referred to as total DNA or eDNA (Taberlet et al. 2012a;Taberlet et al. 2012b; van der Loos and Nijland 2021; Pawlowski et al. 2022), and in this study, as sediment eDNA (SedDNA).

ComDNA extraction concentrates sediment samples into a smaller sample volume through sieving and decantation or centrifugation of sediments, thus reducing the number of non-target organisms and potential PCR inhibitors (Brannock and Halanych 2015; Nascimento et al. 2018) and yielding higher-quality DNA (van der Loos and Nijland 2021). However, the required preprocessing and sieving steps demand considerable time and resources. Conversely, extracting organisms from a larger, homogenized sample can boost species detectability (Pawlowski et al. 2022). It is also important to recognize that these extraction strategies may damage or exclude soft-bodied taxa (Brannock and Halanych 2015; Carugati et al. 2015).

Extracting eDNA directly from sediments is a reliable method for revealing benthic biodiversity (Fais et al. 2020; Lejzerowicz et al. 2021) and has several advantages. It increases the number of samples that can be processed by reducing onboard processing time and minimizes contamination risks (Brandt et al. 2020). Additionally, it can increase the likelihood of detecting a broader range of species, including larger ones, through the presence of eggs, juveniles, or traces of their DNA within the sediment (Aylagas et al. 2016; Atienza et al. 2020). However, eDNA also captures DNA from organisms that may not currently inhabit the sampled sediment, reflecting past or nearby communities (Gielings et al. 2021; Pawlowski et al. 2022). Thus, eDNA provides a broader, less substrate-specific snapshot of benthic diversity, whereas ComDNA offers a more selective view of the organisms physically present in the sediment.

Although eDNA metabarcoding has been used to reveal deep-sea benthic biodiversity (Guardiola et al. 2015; Fonseca et al. 2017), standardized protocols are crucial for the comparability of future metabarcoding studies targeting benthic communities (Suter et al. 2021; Gielings et al. 2021; Girard et al. 2022; van den Bulcke et al. 2023). Key factors, such as the required sediment sample volume to capture an accurate and comprehensive representation of the targeted benthic community, must be thoroughly evaluated (Nascimento et al. 2018; van der Loos and Nijland 2021).

The objective of this study is to compare two different DNA extraction strategies—ComDNA and SedDNA—to assess deep-sea benthic fauna diversity using DNA metabarcoding. The underlying question is whether SedDNA metabarcoding can yield a representation of deep-sea benthic diversity comparable to that obtained from ComDNA sample metabarcoding. Studies directly comparing SedDNA and ComDNA metabarcoding remain rare, underscoring the importance of this research (Macher et al. 2018). Furthermore, an additional aim is to provide recommendations for the sediment sample volume required to capture a representative fraction of benthic diversity, which should be incorporated into future standardized metabarcoding protocols.

Materials and methods

Sample collection and processing

Benthic samples were collected during the MANGAN 2018 expedition, conducted by the Federal Institute for Geosciences and Natural Resources (BGR) aboard the *RV Sonne* as part of the SO262 cruise from 5 April to 29 May 2018.

Short sediment cores were retrieved from the northern part of Working Area 1 (WA-1), located in the eastern part of the BGR contract area for the exploration of polymetallic nodules in the Clarion–Clipperton Fracture Zone (CCZ) of the Northeast Pacific Ocean. Sediment cores were collected at 19 stations using a multicorer (MUC). From each of the 19 deployments, one sediment core was obtained for metabarcoding analyses (Table 1). Each core tube was 60 cm in length, with an inner diameter of 9.6 cm.

For SedDNA metabarcoding, a syringe subsample (1 cm diameter, 2.35 mL) was taken from the top 3 cm of each core (n = 19) and stored at −20 °C. The frozen subsamples were subsequently sliced into 1 cm sections down to 3 cm to analyze the benthic diversity of each layer separately (Fig. 1). To prevent cross-contamination, cutting tools and the workbench were sterilized with 5% bleach between each layer and sample (Lins et al. 2021).

For ComDNA metabarcoding, the extraction of meiofaunal organisms was conducted as follows: the top 3 cm of each sediment core, approximately 217 mL, was sliced and, together with material obtained by filtering overlying water through a 32 µm sieve, stored in DESS, a solution consisting of dimethyl sulphoxide, disodium EDTA, and saturated NaCl (Yoder et al. 2006), at +4 °C. Meiofaunal organisms were extracted from the sediment using the differential flotation method on a colloidal silica gel following McIntyre and Warwick (1984), but using Levasil® (40%) instead of Ludox® and adding three spoons of China clay (kaolinite) to the centrifugation jar. The China clay is a very fine sediment and settles last, creating a compact layer separating the sampled sediment from the Levasil and making it easier to wash the sample without resuspending the sediment. Samples were centrifuged three times using a Heraeus Megafuge at 6,000 rpm for 5 min. The extracted meiobenthos was preserved in DESS and stored at +4 °C until DNA extraction. For more detailed information on station locations, sampling area, and protocols, refer to the SO262 short cruise report (Rühlemann and cruise participants 2019).

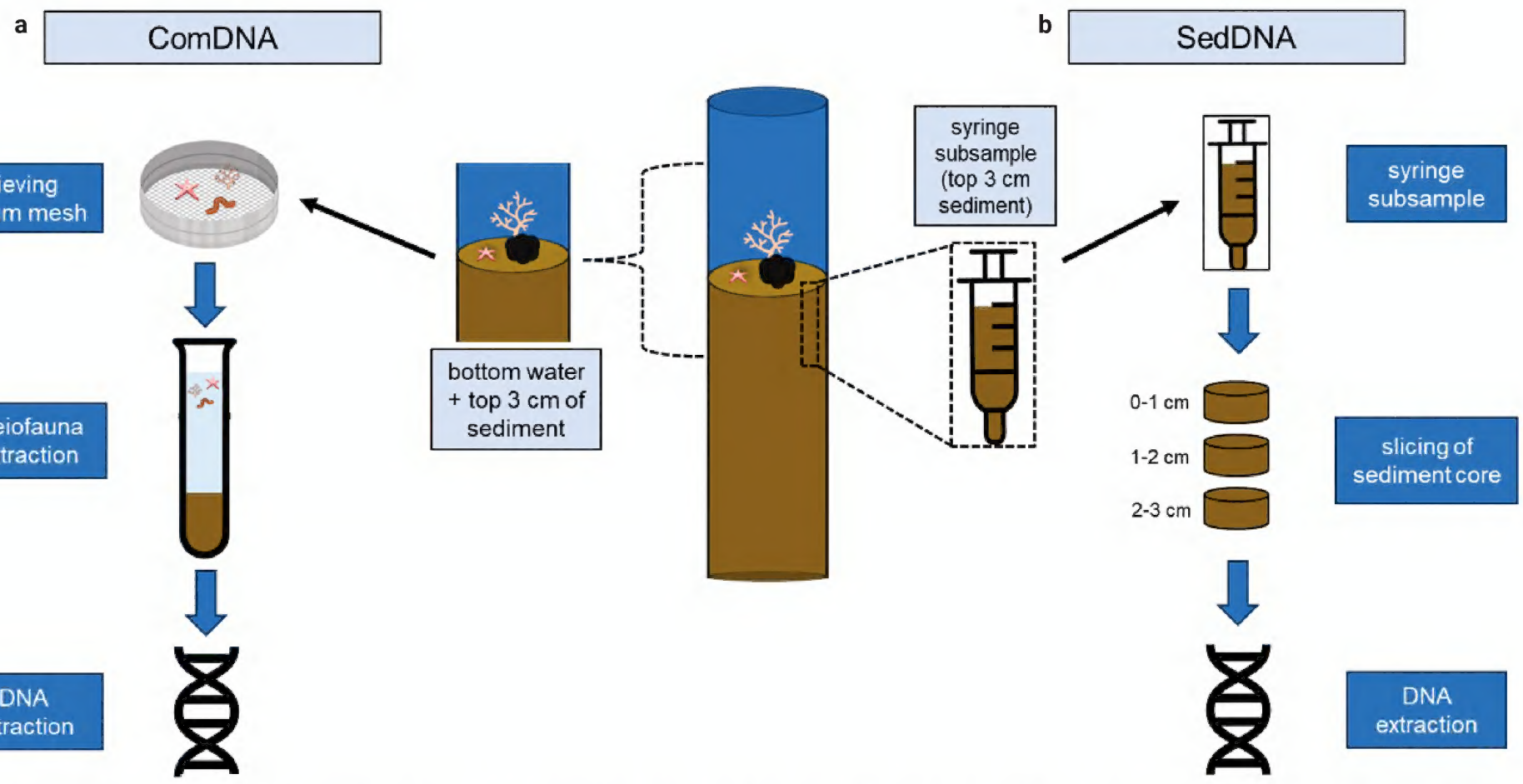

Figure 1. Schematic workflow of the two DNA extraction strategies. (**a**) ComDNA DNA extraction from isolated benthic organisms ("ComDNA") and (**b**) direct total DNA extraction from sediment ("SedDNA").

Table 1. List of the 19 stations in Working Area 1 (WA-1) from which sediment samples were obtained using a multicorer (MUC), including station identifiers, geographic coordinates (latitude and longitude), and water depth.

Station	Date [UTC]	Coordinates	Depth [m]
003MUC	14.04.2018	11°55.782'N, 117°01.591'W	4082
004MUC	14.04.2018	11°55.786'N, 117°01.317'W	4092
009MUC	15.04.2018	11°56.629'N, 117°01.811'W	4097
010MUC	15.04.2018	11°56.855'N, 117°01.106'W	4080
011MUC	15.04.2018	11°57.056'N, 117°00.538'W	4014
013MUC	15.04.2018	11°56.210'N, 117°01.895'W	4083
016MUC	15.04.2018	11°56.129'N, 117°01.436'W	4079
017MUC	16.04.2018	11°56.419'N, 117°01.174'W	4077
021MUC	17.04.2018	11°55.691'N, 117°00.955'W	4090
023MUC	17.04.2018	11°55.257'N, 117°01.579'W	4081
024MUC	17.04.2018	11°55.510'N, 117°01.246'W	4091
025MUC	17.04.2018	11°55.526'N, 117°00.733'W	4095
026MUC	17.04.2018	11°55.320'N, 117°00.435'W	4102
027MUC	17.04.2018	11°55.042'N, 117°00.634'W	4098
033MUC	18.04.2018	11°55.954'N, 117°00.819'W	4092
034MUC	19.04.2018	11°56.140'N, 117°01.225'W	4075
038MUC	19.04.2018	11°56.178'N, 117°00.804'W	4091
042MUC	20.04.2018	11°56.833'N, 117°00.127'W	4109
043MUC	20.04.2018	11°56.850'N, 116°59.983'W	4107

DNA extraction and NGS library preparation

DNA extraction

DNA from ComDNA samples was extracted using the E.Z.N.A.® Mollusc & Insect DNA Kit (Omega Bio-tek) according to the manufacturer's instructions. Prior to DNA extraction, the DESS-fixed meiofauna were filtered using sterile glass filters with a particle size of 2.7 µm (Whatman) to extract the meiofauna. Ethanol (denatured 96%) was used to wash the filter and remove the DESS residues. Each filter containing the meiofauna was transferred to a separate DNA-free 1.5 mL Eppendorf tube and further dried using a speed-vacuum system at 45 °C for 1 hour (Eppendorf™ Concentrator Plus). For the SedDNA samples, eDNA was extracted from sediment using the DNeasy® PowerSoil® Pro Kit (Qiagen), following the manufacturer's protocol with modifications to enhance DNA yields. For each layer, 0.785 mL of wet sediment was processed. Due to the high amount of water, sediment samples were dried in a speed-vacuum at 45 °C for 3 hours (Eppendorf™ Concentrator Plus). Homogenization was performed using a tissue homogenizer (BeadBlaster™ 24, Benchmark Scientific) for 10 cycles of 1 min at 5 m/s. DNA was eluted using 50 µL of Solution CD6, incubated at room temperature for 1 min, and then centrifuged first at 1,000× g for 1 min, followed by 15,000× g for 1 min. To maximize DNA yield, the 50 µL flow-through was reapplied to the same column, followed by repeated incubation and centrifugation. Extracted DNA was quantified with a Qubit™ dsDNA HS assay (Invitrogen™ Qubit™ 3.0 fluorometer, Thermo Fisher Scientific).

Amplicon library preparation and sequencing

For DNA metabarcoding intended for Illumina MiSeq sequencing, the ~350 bp long hypervariable V1–V2 region of the nuclear small subunit (nSSU) 18S rRNA gene (Fonseca et al. 2014) was amplified using the universal SSU_F04 (5'-GCTTGTCTCAAAGATTAAGCC-3') and SSU_R22 (5'-GCCTGCTGCCTTCCTTGGA-3') primer pair (Blaxter et al. 1998). These primers were tagged with Nextera XT Illumina adapters (forward adapter: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'; reverse adapter: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3') according to the 16S Metagenomic Sequencing Library Preparation guide (15044223-b). ComDNA PCR amplification of the target gene fragment was carried out on an Eppendorf thermocycler as follows: initial denaturation at 98 °C for 2 min, followed by 25 cycles of 98 °C for 15 s, 57 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. A two-step qPCR was then conducted to bind the Illumina Unique Dual Nextera Indexes and compatible adapter overhangs to the amplicons (IDT® for Illumina® UD indexes) at a final volume of 20 µL. The cycler settings included denaturation at 98 °C, followed by annealing and elongation at 72 °C for 45 s for 10 cycles. Amplifications were performed using the Phusion™ High-Fidelity DNA Polymerase (Thermo Fisher Scientific), and the PCR products were checked on a 1.5% agarose gel containing 1% GelRed to ensure the amplicon fragment size.

For SedDNA, a CFX69™ Real-Time System (C1000 Touch™, Bio-Rad) was used to perform the PCRs. The locus-specific qPCR was conducted in a total volume of 20 µL, including 10 µL SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), 1 µL of each primer (10 pM/µL), 2–8 µL genomic DNA, and 0–6 µL molecular-grade water. The qPCR cycling settings consisted of an initial denaturation step at 98 °C for 2 min, followed by 25 cycles of denaturation at 98 °C for 15 s, annealing at 57 °C, and elongation at 72 °C for 1 min. A two-step qPCR was then conducted to bind the indexes, including denaturation at 98 °C, followed by annealing and elongation at 72 °C for 45 s for 10 cycles. Amplification success, together with the Cq values and relative fluorescence units (RFU), was assessed by inspecting the qPCR amplification curves. Both assays also included a melting-temperature analysis (65–99 °C) to verify denaturation peak profiles. Each PCR plate included a no-template PCR negative control and a PCR positive control consisting of a synthetic gene fragment containing the primer-binding regions of the target locus. Both controls were amplified and sequenced alongside all environmental samples. Reads originating from the synthetic positive control were removed bioinformatically before downstream analyses. PCR amplification was performed without technical replicates.

The indexed PCR products from the ComDNA and SedDNA samples were pooled separately at equimolar concentrations, resulting in two multiplexed libraries, one per method. Each pooled library was purified using Agencourt AMPure XP magnetic beads (Beckman Coulter) with size selection. Library quantification was performed using both the Collibri™ Library Quantification Kit (Thermo Fisher Scientific) and the Qubit™ dsDNA HS Assay (Invitrogen, Thermo Fisher Scientific), following the manufacturers' protocols.

Prior to high-throughput sequencing, the quality and normalization of each multiplexed library were assessed with a MiSeq v2 Nano kit (1 million reads, paired end, 250 cycles). The two normalized libraries were then sequenced separately, each using a MiSeq v3 kit (25 million reads, 300 cycles, paired end), at the Senckenberg am Meer Metabarcoding and Molecular Laboratory (DZMB, Wilhelmshaven, Germany).

Sequence reads processing and taxonomic assignment

Sequence data processing was conducted on the high-performance computing cluster (HPC) of the RPTU Kaiserslautern–Landau. Quality checks of the Illumina R1 and R2 raw sequence reads were carried out using FastQC v0.12.1 (Andrews 2010) and summarized with MultiQC v1.16 (Ewels et al. 2016). Primer sequences were removed using BBDuk v39.01 (DOE Joint Genome Institute, Berkeley, California, USA).

The dada2 (divisive amplicon denoising algorithm) v1.16 workflow (Callahan et al. 2016) was executed in R v4.3.3 (R Core Team 2024) within RStudio v2024.4.2.764 (Posit team 2024) to quality-filter and trim the raw sequence reads, generating amplicon sequence variants (ASVs). Read truncation was performed using the truncQ = 30 parameter, truncating reads at the position where the mean Phred quality score dropped below Q30, thereby retaining only bases with ≥ 99.9% base-call accuracy (Ewing and Green 1998; Stoeck et al. 2024). The maxEE parameter was set to 1 to maintain downstream sequence quality. Paired-end sequences were dereplicated and merged with a minimum 20 bp overlap and a maximum mismatch of two bases (Frühe et al. 2021). Chimera detection and removal were performed using the *uchime-denovo* function (Edgar et al. 2011) of vsearch v2.15.2 (Rognes et al. 2016). The final ASV-to-sample matrix was generated by removing ASVs represented by only a single sequence across the entire dataset (singletons) (Stoeck et al. 2024) and by removing non-metazoan taxa.

A custom script (SGN Metabarcoding Pipeline; https://github.com/pmartinezarbizu/Methods_Metabarcoding-meiofauna_2023) was used to align ASVs against the NCBI database (Benson et al. 2013) using the BLAST tool (Altschul et al. 1997) within the blastn v2.15.0 pipeline. The top 10 BLAST hits were retrieved and merged with the V1–V2 barcode reference archive of DZMB (Senckenberg am Meer, Wilhelmshaven, Germany). This combined dataset served as the final custom BLAST database, enabling the most accurate taxonomic assignment for each ASV. The assignments included percentage identity, query coverage, fragment length, GenBank accession number, and e-value. Before clustering the ASVs into operational taxonomic units (OTUs), samples with fewer than 10,000 reads were excluded from the dataset. This filtering step resulted in the exclusion of five SedDNA samples (03_1, 09_2, 13_3, 23_2, and 27_2) and one ComDNA sample (sample 24). Subsequently, both datasets were rarefied (normalized) to the smallest sample size of 15,005 sequences (SedDNA 33_1) using the *rrarefy* function from the vegan v2.6.4 package (Oksanen et al. 2022). The taxonomic assignments of the ASVs were cross-checked against WoRMS—World Register of Marine Species (WoRMS Editorial Board 2024)—to obtain the most up-to-date status, using the taxFromWorms function of the *dada2pp* package (Martinez Arbizu 2018) of R. The rarefied

ASVs were subsequently aligned using the *AlignSeqs* function of the DECIPHER (Wright 2024) package in R, and the *IdClusters* function was used to calculate the genetic distance matrix. The ASVs were then clustered into OTUs using a neighbor-joining approach with a 3% similarity threshold, implemented with the *aggregateASV* function of the *dada2pp* package (https://github.com/pmartinezarbizu/dada2pp). OTU clustering was applied to reduce diversity inflation arising from sequencing artefacts. The use of a 97% similarity cut-off is common in metabarcoding studies targeting benthic communities (Fonseca et al. 2017; Gielings et al. 2021; Liu and Zhang 2021), and OTU clustering continues to be recommended to mitigate artefactual richness inflation (Lejzerowicz et al. 2021). The rarefied OTU dataset of combined ComDNA and SedDNA samples was used for diversity and community composition analyses. The 0–3 cm layers from each SedDNA sample were pooled to match the 0–3 cm section used for ComDNA, ensuring comparability between the two extraction strategies. In addition, it was verified that OTU composition did not differ significantly between the individual 1 cm sediment layers prior to pooling.

For the estimation of the sediment volume required to achieve OTU richness comparable to ComDNA samples, OTU clustering was also performed on the non-rarefied ASV table to retain the full sequencing depth. Due to the removal of the *IdClusters* function in recent versions of DECIPHER, the *Clusterize* function from the DECIPHER v2.30.0 package was used, applying the same 3% similarity threshold. The resulting OTU tables were then rarefied to 50,000 and 100,000 reads using the *rrarefy* function from the vegan v2.6.4 package to assess the effect of sequencing depth on OTU richness. To preserve the full sequencing depth, one dataset was left unrarefied.

Statistics

Statistical analyses and graphical visualizations were performed in R v4.3.3 (R Core Team 2024) within RStudio v2024.4.2.764 (Posit team 2024), using the packages vegan v2.6.6.1 (Oksanen et al. 2022), tidyverse v2.0.0 (Wickham et al. 2019), stats v.4.3.3 (R Core Team 2024), ggplot2 v3.5.1 (Wickham 2016), and dplyr v1.1.4 (Wickham et al. 2023).

The rarefied OTU richness, the Simpson diversity index (D) (Simpson 1949), and the Shannon–Wiener index (H') (Shannon and Weaver 1949) were calculated from the OTU-to-sample matrix as measures of alpha diversity to compare the metazoan communities across different sediment layers and between the two DNA extraction strategies. The normality of the datasets was assessed using the Shapiro–Wilk test (Shapiro and Wilk 1965), computed with the *shapiro.test* function, while homogeneity of variance was evaluated using Levene's test (Levene 1960) with the *levene.test* function. To test for significant differences in alpha diversity between sediment layers and extraction strategies, the Kruskal–Wallis rank-sum test (Kruskal and Wallis 1952) was performed using the *kruskal.test* function of the stats package. A post hoc pairwise Wilcoxon rank-sum test (Wilcoxon 1945) with Benjamini–Hochberg p-value adjustment (Benjamini and Hochberg 1995) was performed using the *pairwise.wilcox.test* function. The abundance-based Bray–Curtis (BC) index (Bray and Curtis 1957), as well as the presence–absence-based Jaccard index, were calculated as measures of beta diversity based on the fourth-root-transformed OTU reads

using the *vegdist* function of the vegan package. Non-metric multidimensional scaling (nMDS) was employed to visualize metazoan community similarities across the separate layers of SedDNA samples and to compare community similarities across all samples from the two extraction strategies.

To assess differences in community composition between SedDNA sediment layers and between SedDNA and ComDNA samples, pairwise PERMANOVA tests (permutational multivariate analysis of variance) were performed using the *pairwise.adonis2* function from the pairwiseAdonis v0.4.1 package (Martinez Arbizu 2020). Longitude, latitude, and extraction method were included as factors to evaluate their influence on community dissimilarity. For the SedDNA dataset, the sediment layer was also included as a factor to determine whether sediment depth affects the composition of benthic communities. Because sediment layers (depths) were sampled repeatedly within the same cores and were therefore not fully independent, the *strata* argument was used to control for the effect of individual liners (cores). By specifying core ID as the stratification factor, permutations were restricted within each core, appropriately accounting for the nested structure of the data.

Mantel tests (Mantel 1967) were conducted to evaluate significant correlations between distances of metazoan communities across all samples, including both SedDNA separate layers and ComDNA samples, and the geographic distances between sampling stations. Distance matrices were derived using Bray–Curtis distances for both SedDNA separate layers and ComDNA samples. The geographic distance matrix was calculated based on the coordinates of the sampling stations (Rühlemann and cruise participants 2019) using the *distm* function of the geosphere v1.5.18 package (Hijmans et al. 2024). Final Mantel tests were performed using the *mantel* function of the vegan package, based on Spearman's rho with 9,999 permutations. Venn diagrams were created using the VennDiagram v1.7.3 package (Chen and Boutros 2011) to illustrate shared OTUs between the SedDNA and ComDNA samples.

Estimation of required sample size for SedDNA metabarcoding

A random subsampling of sample combinations (without replacement) was applied to determine the OTU richness levels for different combinations of SedDNA sample sizes. This enabled the estimation of the number of samples, and consequently the sediment volume, required to achieve OTU richness levels from SedDNA samples comparable to those from ComDNA samples.

The dataset used for the main biodiversity analyses of this study, including diversity measures and taxonomic community composition, was rarefied at the ASV level to 15,005 reads per layer using the *rrarefy* function from the *vegan* package, following pooling of the sediment layers. After clustering ASVs into OTUs, the data were then down-sampled to 6,680 reads, corresponding to the lowest read count of the ComDNA dataset (sample 17). This dataset served as the standardized basis for direct comparisons between SedDNA and ComDNA samples. To further examine the required SedDNA sample size for achieving OTU richness levels comparable to ComDNA samples under different sequencing depths, based on metazoan reads only, additional dataset treatments were included: a dataset rarefied to 50,000 reads, a dataset rarefied to 100,000 reads, and a non-rarefied dataset.

These additional datasets were used specifically to explore how sequencing depth affects the estimated SedDNA sample size required to capture metazoan OTU richness levels comparable to those of ComDNA samples and serve as a basis for providing methodological recommendations for future studies applying SedDNA metabarcoding.

For all datasets, a down-sampling approach was then applied to avoid artificially inflating sequencing depth with increasing combination sizes. With a total of 19 samples, subsamples were generated sequentially by dividing the maximum number of reads by the number of samples (x/1–x/19), ensuring consistent read counts across all sample combinations (1×–19×). For the non-rarefied dataset, no down-sampling or rarefaction was applied; instead, 19 identical samples containing the full read counts were used.

The final determination of the SedDNA sample size required to resemble the OTU richness of the ComDNA samples was conducted using random subsampling of sample combinations (without replacement). The number of possible combinations of the 19 samples was calculated using the following equation:

$$combinations = \frac{n!}{(n-k)! * k!} = \binom{n}{k} \quad \text{Eq. 1}$$

where *n* is the total number of samples and *k* is the number of random samples combined. A maximum of 1,000 iterations were performed for random combinations of samples where *k* = 4 to *k* = 15. Values for combinations with *k* = 1 to *k* = 3 and *k* = 16 to *k* = 19 were obtained separately, as they had fewer possible combinations than 1,000 iterations. To estimate the number of SedDNA samples needed, both a linear model and a model based on the Michaelis–Menten (MM) equation were applied. The MM model was chosen because saturation in the SedDNA OTU richness levels was expected with increasing sample combinations. The required sample volume was then determined by multiplying the required sample size by the processed sample volume.

Using the linear model, the mean OTU richness of the ComDNA samples was calculated as follows:

$$mean\ ComDNA\ OTU\ richness = \beta_0 + \beta_1 * combinations \quad \text{Eq. 2}$$

where β0 is the intercept of the regression line, and β1 is the slope of the regression line. The required sample size for SedDNA samples was given by:

$$required\ sediment\ sample\ size = \frac{ComDNA\ OTUs - \beta_0}{\beta_1} \quad \text{Eq. 3}$$

where β0 is the intercept of the regression line, and β1 is the slope of the regression line.

Additionally, the required SedDNA sample size was estimated using the Michaelis–Menten model (nonlinear least squares, NLS) to account for the expected saturation in OTU richness levels. The mean OTU richness of the ComDNA samples was calculated according to the following equation:

$$mean\ ComDNA\ OTU\ richness = \frac{V_{max} * X}{(K_m + X)} \quad \text{Eq. 4}$$

where K_m represents the Michaelis constant (the sample size at which OTU richness is half of V_{max}), V_{max} is the maximum OTU richness, and X is the sample size. To determine the required SedDNA sample size, the Michaelis–Menten equation was rearranged as follows:

$$required\ sediment\ sample\ size = \frac{(K_m * ComDNA\ OTUs)}{(V_{max} - ComDNA\ OTUs)} \quad \text{Eq. 5}$$

Here, K_m and V_{max} are the estimated values obtained from the NLS, and OTUs represent the mean OTU richness of the ComDNA samples.

The required sediment volume was then determined by multiplying the calculated required SedDNA sample size by the processed volume of SedDNA samples and is given by:

$$required\ sediment\ volume = required\ sediment\ sample\ size * processed\ sample\ volume \quad \text{Eq. 6}$$

Results

Sequence data overview

After quality filtering, merging, chimera removal, and singleton removal, a total of 3,619,029 and 6,785,050 high-quality (HQ) reads were retained in the ComDNA dataset and the SedDNA dataset, respectively. Following taxonomic assignment, all non-metazoan reads were removed, and only metazoan sequences were retained for downstream analyses. The negative PCR controls contained only non-target sequences (e.g., human, terrestrial insects, and non-marine bacteria), all of which were removed during the taxonomic filtering step. After filtering, no reads remained in any negative control, indicating the absence of detectable cross-contamination. Clustering the ASVs into OTUs yielded 2,145 OTUs for the ComDNA dataset and 392 OTUs for the SedDNA dataset. In total, 227,610 reads corresponding to 2,145 OTUs were retained for the ComDNA dataset, while 79,298 reads corresponding to 392 OTUs were retained for the SedDNA dataset. Three SedDNA samples (17_2, 23_2, and 38_1) were excluded from downstream analyses due to the absence of OTUs.

Statistical analysis of diversity metrics

OTU richness, the Shannon index, and Simpson diversity indices did not differ significantly among the three SedDNA layers (Fig. 2). Likewise, non-metric multidimensional scaling (nMDS) based on Bray–Curtis distance revealed highly similar metazoan communities across the layers (Suppl. material 1: fig. S1). Because no meaningful variation in diversity metrics was detected, separate analyses by layer were deemed unnecessary. Notably, samples 04_2, 11_2, and 16_3 each yielded only a single OTU, preventing the calculation of alpha diversity indices for these samples. Consequently, all subsequent analyses were conducted on the combined SedDNA layer.

Pairwise Wilcoxon tests confirmed that OTU richness was significantly greater in the ComDNA dataset ($p < 0.01$) and that Shannon index values followed the same pattern ($p < 0.05$), while no significant difference was detected for Simpson

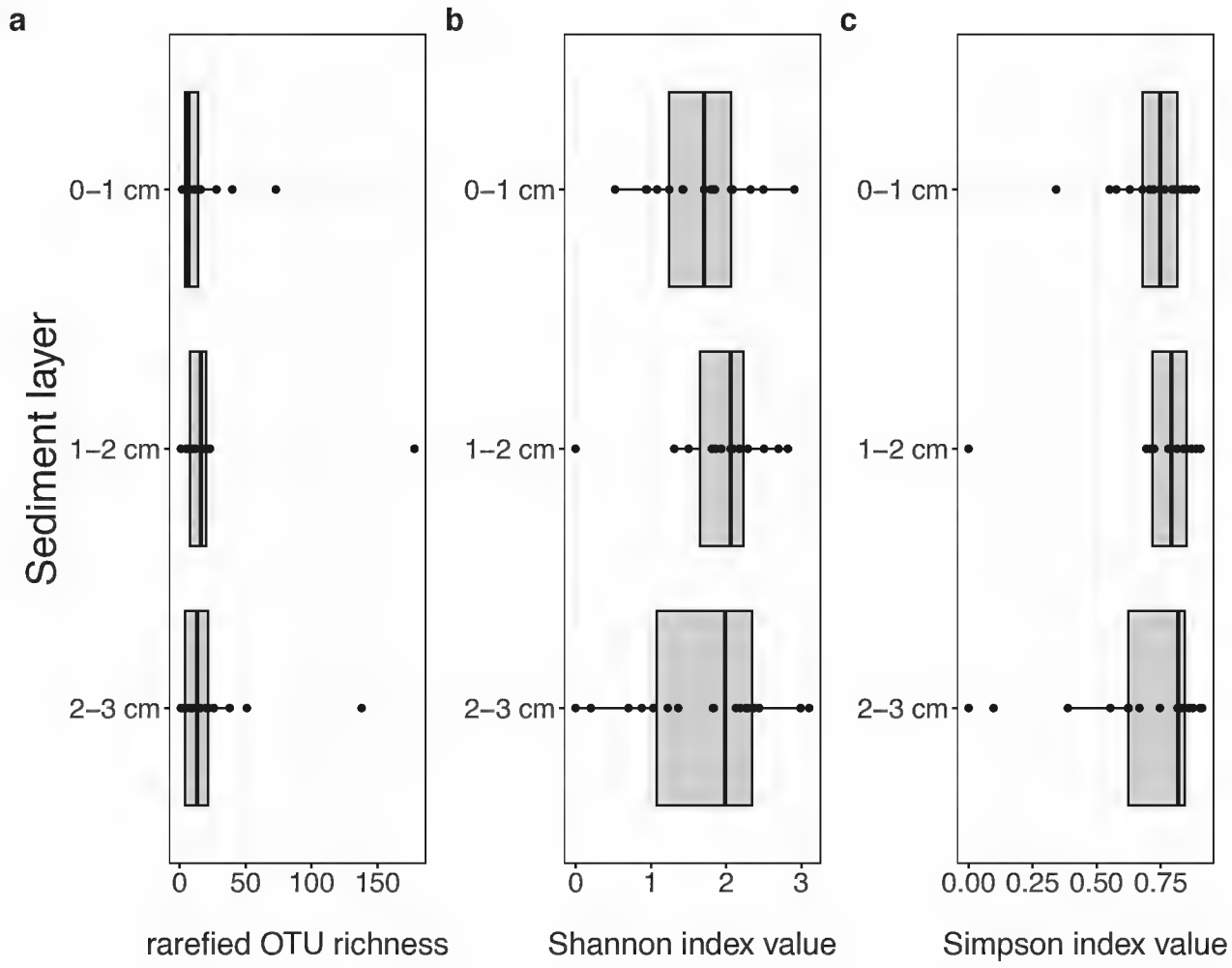

Figure 2. Alpha diversity of benthic metazoans across sedimentary eDNA (SedDNA) layers. Boxplots showing measures of alpha diversity of benthic metazoan communities across the different SedDNA layers: **a**. Rarefied OTU richness; **b**. Shannon diversity index, and **c**. Simpson diversity index. Boxes show the interquartile range (25th–75th percentile) with the median as a horizontal line; whiskers extend to 1.5 × IQR, and points beyond this range are outliers.

index values (Fig. 3). In the ComDNA dataset, OTU richness ranged from 187 OTUs (sample 34) to 430 OTUs (sample 21), whereas in the SedDNA dataset, rarefied OTU richness ranged from 8 OTUs (sample 09) to 232 OTUs (sample 03). Species accumulation curves (Fig. 3d) additionally illustrate that ComDNA samples show a much higher accumulation of OTUs compared with SedDNA samples. Furthermore, initial analyses at the ASV level confirmed the same patterns observed with OTUs: ComDNA exhibited a significantly higher ASV richness compared with SedDNA samples ($p < 0.001$; Suppl. material 1: fig. S5).

Estimation of required sediment sample size

Because SedDNA OTU richness was substantially lower than that observed in ComDNA samples, we applied a random subsampling of sample combinations (without replacement) (1,000 iterations), combining between one and nineteen randomly selected SedDNA samples to model OTU accumulation (Suppl. material 1: fig. S2). Based on these results, both a linear model (Eq. 2) and a Michaelis–Menten (MM) model were fitted to the resulting richness curves to estimate the number of SedDNA samples, and thus the sediment volume, required to reach OTU richness levels comparable to those obtained from ComDNA metabarcoding. The target OTU richness was defined as the observed mean richness of the ComDNA samples and varied depending on the dataset processing approach. Specifically, the target richness was 266 OTUs for the dataset rarefied at the ASV level, 332 OTUs for the dataset rarefied to 50,000 reads, 353 OTUs for the dataset rarefied to 100,000 reads, and 367 OTUs for the non-rarefied dataset (Table 2).

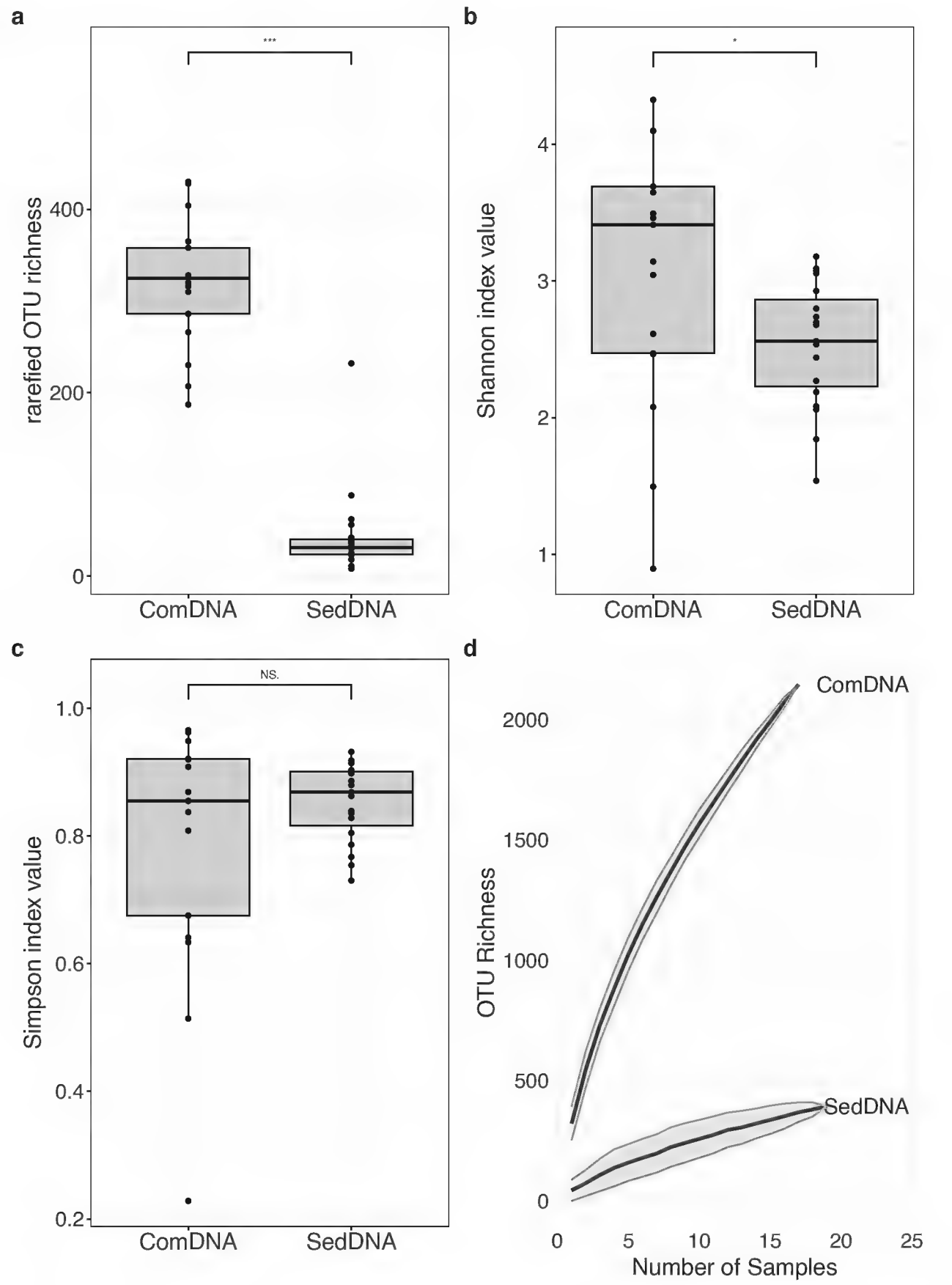

Figure 3. Alpha diversity metrics and species accumulation in SedDNA and ComDNA samples. Boxplots depicting rarefied OTU richness (**a**), Shannon index (**b**), Simpson index (**c**), and species accumulation curves (**d**) for benthic metazoan communities from SedDNA and ComDNA extractions. Boxes show the interquartile range (25th–75th percentile) with the median as a horizontal line; whiskers extend to 1.5 × IQR, and points beyond this range are outliers. Asterisks indicate statistically significant differences (pairwise Wilcoxon test): *p < 0.05, **p < 0.01, ***p < 0.001; NS = not significant.

For the linear model (Eq. 3), the required number of SedDNA samples to reach the respective target OTU richness was calculated based on the maximum and minimum observed OTU richness levels from the bootstrap results. Here, maximum OTU richness refers to the highest OTU count detected in any combination of SedDNA samples, whereas minimum OTU richness corresponds to the lowest OTU count observed. This approach allows us to estimate a range of required sample sizes and sediment volumes, reflecting the variability in OTU richness due to random subsampling. Using this range provides a more conservative and realistic estimate of the sampling effort needed to match the

biodiversity captured by ComDNA samples. Sediment volumes were derived by multiplying the estimated number of samples by the per-sample processed volume (2.35 mL). All resulting volume estimates for the ASV-level dataset and the datasets rarefied to 50,000 reads, 100,000 reads, and the non-rarefied dataset are summarized in Table 2.

A Michaelis–Menten model was additionally applied to account for potential richness saturation with increasing numbers of combined SedDNA samples. Although no clear plateau was reached within the sampling range, the rearranged MM equation (Eq. 5) allowed estimation of the required number of samples, which were again converted to sediment volume. The corresponding volume estimates for all datasets are likewise provided in Table 2.

By comparison, the ComDNA metabarcoding protocol processed 217 mL of sediment for organism extraction (Eq. 6).

Statistical and taxonomic comparison of SedDNA vs. ComDNA

nMDS based on both Bray–Curtis and Jaccard distances revealed two distinct benthic communities corresponding to the two DNA extraction strategies (Fig. 4). Similarly, nMDS ordination based on ASV-level Bray–Curtis dissimilarity showed two distinct, non-overlapping communities corresponding to ComDNA and SedDNA samples (Suppl. material 1: fig. S6).

A pairwise PERMANOVA (Suppl. material 1: table S1) on individual SedDNA layers (0–1 cm, 1–2 cm, 2–3 cm) and sampling coordinates (longitude and latitude) revealed no significant effect of either sediment depth or sampling location. In contrast, when analyzing the full dataset (combined SedDNA layers plus ComDNA samples), the extraction strategy exhibited a highly significant effect ($p < 0.001$), whereas sampling location remained non-significant. These results mirror the separation seen in the nMDS plots (Fig. 4).

To qualitatively compare the performance of the two extraction strategies, the taxonomic composition of metazoan communities was evaluated. A detailed phylum-level breakdown of each SedDNA layer is provided in the Suppl. material 1: fig. S4. Although the primary aim of this study was to compare meiofaunal communities, we also retained a few larger, partially benthic groups, such as Chaetognatha, Bryozoa, Nemertea, Annelida, and some other rare groups such as Porifera and Cnidaria, whose adults often exceed the typical meiofauna size range but either spend their full life cycle on or near the seafloor or possess

Table 2. Estimated sediment sample volumes (mL) required for SedDNA to reach the observed mean OTU richness of the ComDNA samples. The first column specifies the respective dataset variants used in the analysis. The subsequent columns report (1) required sediment volume at the maximum OTU richness estimate (linear model), (2) required sediment volume at the minimum OTU richness estimate (linear model), (3) required sediment volume based on the Michaelis–Menten (MM) model, and (4) the ComDNA target richness used for each dataset processing method.

Dataset processing method	Required sample volume [mL] at max OTU richness (linear model)	Required sample volume [mL] at min OTU richness (linear model)	Required sample volume [mL] (Michealis-Menten model)	Target OTUs
rarefied on ASV level	46.53	54.05	82.25	266
rarefied to 50K	31.96	49.115	44.18	332
rarefied to 100K	27.025	48.175	38.775	353
not rarefied	12.455	44.18	25.85	367

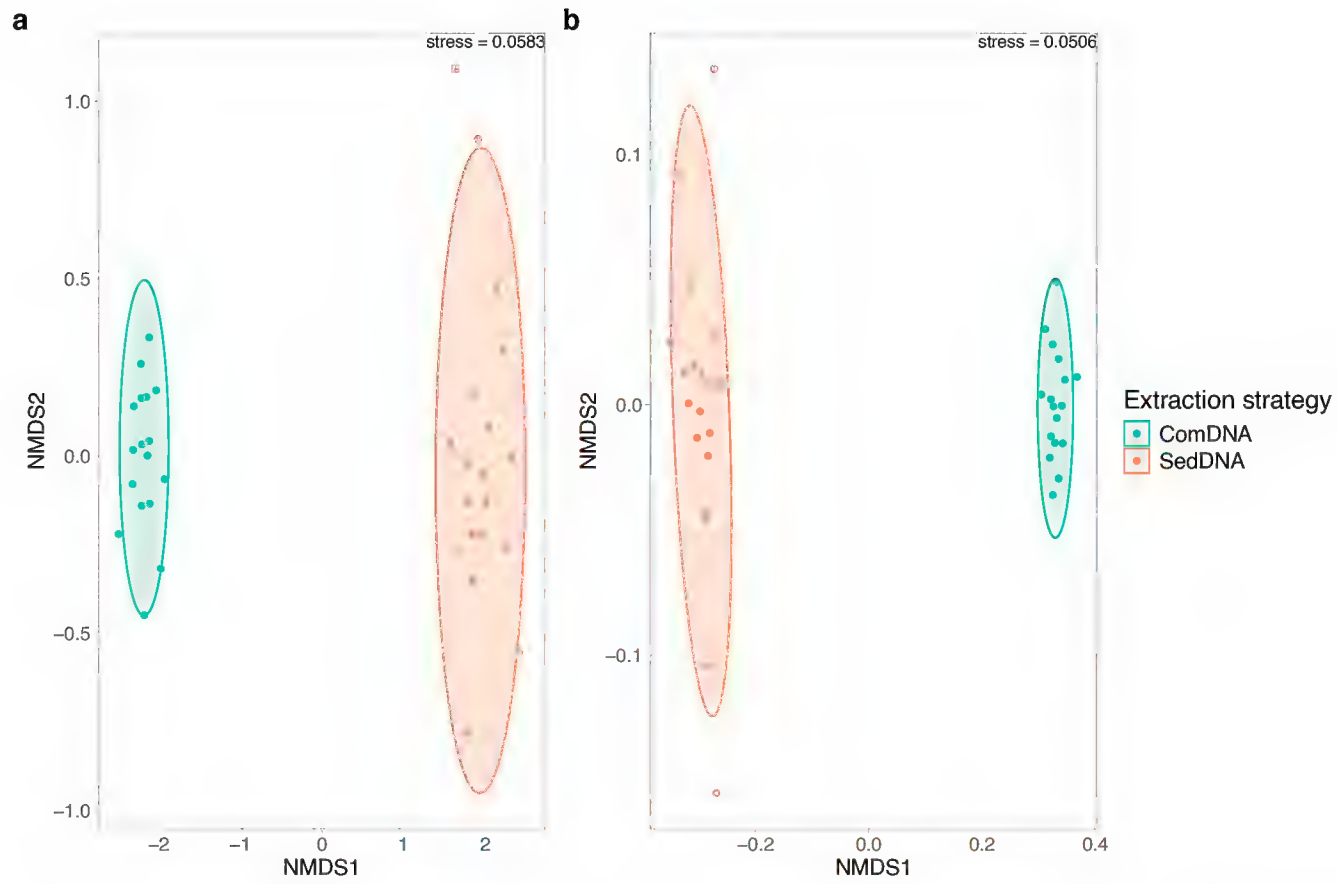

Figure 4. nMDS of benthic community dissimilarity between SedDNA and ComDNA samples. nMDS plots based on (**a**) Bray–Curtis and (**b**) Jaccard distances, illustrating differences in benthic community composition between SedDNA and ComDNA samples. Ellipses indicate 95% confidence intervals.

planktonic larval stages. By including these taxa, we avoided additional post hoc filtering and ensured an unbiased comparison of community composition between the two DNA extraction strategies.

Within the ComDNA dataset, 19 phyla were detected, compared to 15 phyla in the SedDNA dataset. Based on relative read abundance, the most dominant phylum in both datasets was Annelida, comprising 50.5% of all metazoan reads in the ComDNA dataset and 58.8% in the SedDNA dataset (Fig. 5a). In the ComDNA dataset, Nematoda accounted for 23.2% of reads, followed by Arthropoda (14.3%) and Nemertea (6.5%), with the remaining 5.5% distributed across 15 other phyla. In contrast, the second and third most abundant phyla in the SedDNA dataset were Arthropoda (19.5%) and Nematoda (6.7%), followed by Platyhelminthes (3.4%). A detailed per-sample breakdown of relative read abundance at the phylum level is provided in the appendix (Suppl. material 1: fig. S3). Between the two datasets, 15 phyla were shared. Notably, four phyla were detected exclusively in the ComDNA samples—Hemichordata, Loricifera, Chaetognatha, and Entoprocta—each contributing less than 0.23% to the total relative read abundance.

When considering OTU richness, a different pattern emerged for the ComDNA dataset (Fig. 5b), where Nematoda accounted for 37.6% of metazoan OTUs, followed by Arthropoda (24.3%) and Annelida (22.6%). In contrast, the SedDNA dataset showed no change in the order of dominant phyla based on OTU richness, with Annelida (25.4% of metazoan OTUs), Arthropoda (20.1%), and Nematoda (18.5%) remaining the top three groups. Although Annelida contributed over 50% to the relative read abundance in both datasets, their relative contribution to OTU richness was notably lower for both extraction strategies.

Building on the phylum-level results, a markedly different pattern emerged at the OTU level. In total, 2,507 metazoan OTUs were detected across both extraction strategies, yet the vast majority were strategy-specific: 2,115 OTUs

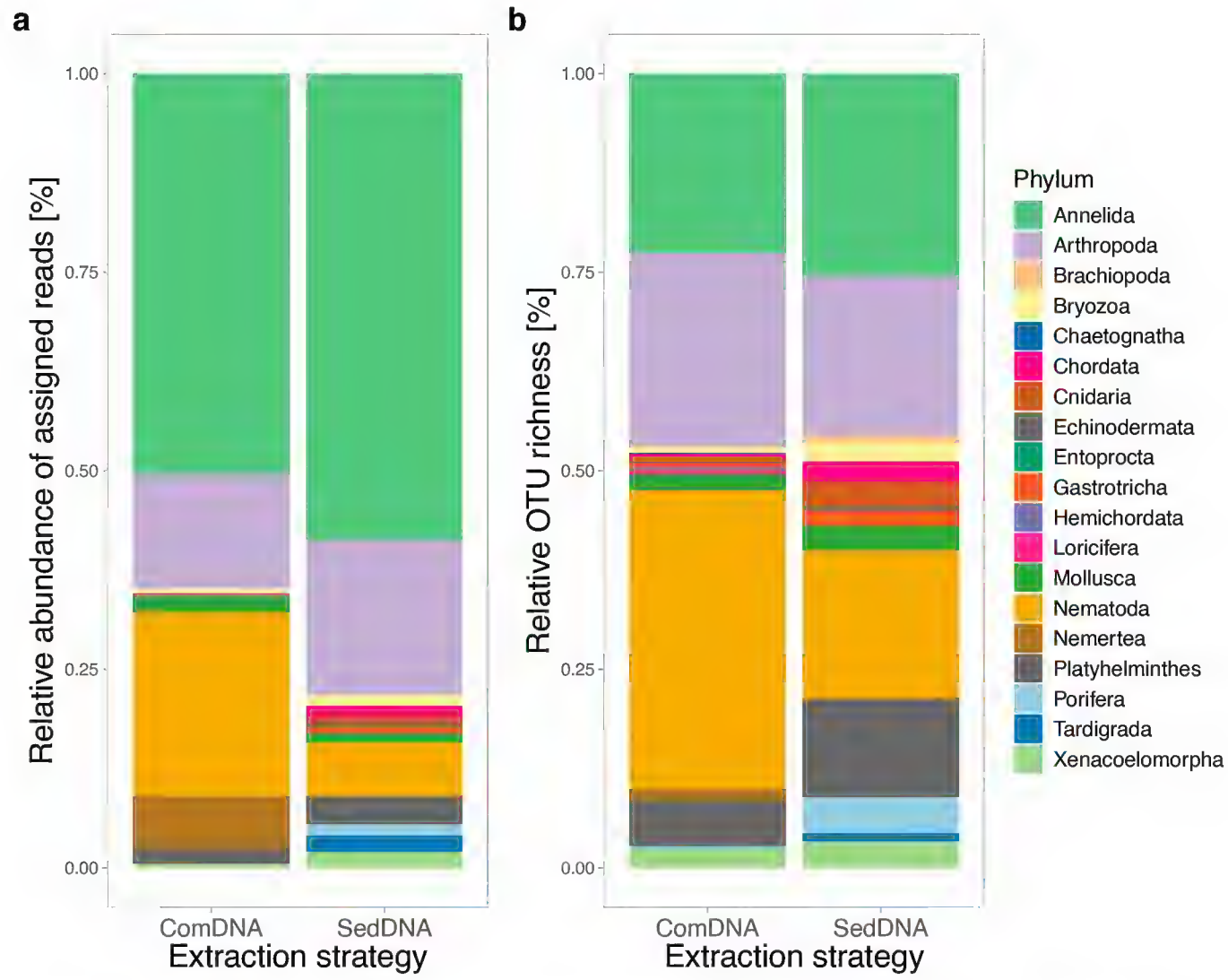

Figure 5. Benthic community structure revealed by SedDNA and ComDNA extraction strategies. Taxonomic composition at the phylum level, showing (**a**) relative read abundance of metazoan phyla and (**b**) relative OTU richness differences between the two extraction strategies.

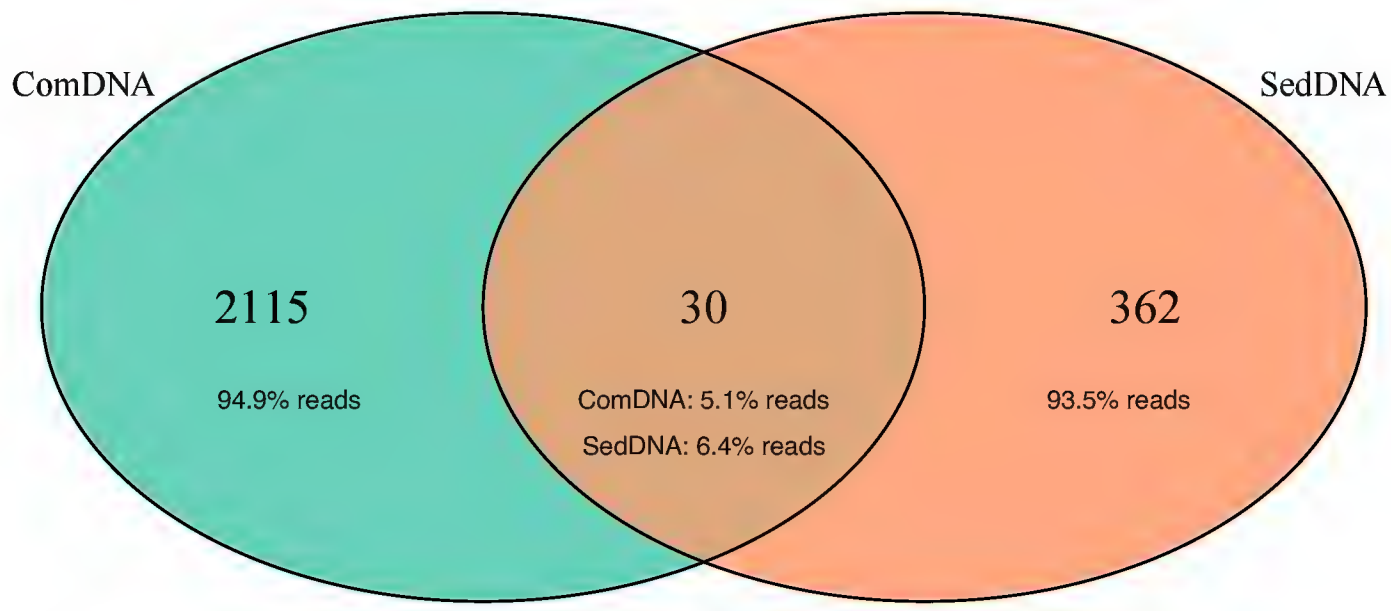

Figure 6. Shared and unique OTUs detected by SedDNA and ComDNA extraction strategies. Venn diagram showing the number of OTUs unique to each extraction strategy and those shared between SedDNA and ComDNA. Percentages indicate the relative proportion of reads contributed by these OTUs within each dataset.

(84.3%) were unique to the ComDNA dataset, and 362 OTUs (14.4%) appeared only in the SedDNA dataset (Fig. 6). Only 30 OTUs (1.2%) were shared by both. Fig. 6 additionally shows the relative read proportions contributed by OTUs within each dataset. In the ComDNA dataset, 2,115 unique OTUs accounted for 94.9% of metazoan reads, while the 30 OTUs shared with SedDNA represented only 5.1%. Similarly, in the SedDNA dataset, 362 unique OTUs contributed 93.5% of metazoan reads, with shared OTUs making up 6.4%. This pronounced segregation aligns with the distinct clustering seen in our nMDS analyses.

Discussion

Methodological differences and implications for biodiversity assessment

Our study highlights significant differences between ComDNA extraction from isolated metazoan organisms and direct sediment eDNA (SedDNA) extraction, emphasizing distinct methodological strengths and limitations. The marked differences observed in OTU richness, with ComDNA extraction detecting 2,145 metazoan OTUs compared to 392 OTUs from SedDNA, underscore previous findings where organismal enrichment procedures effectively concentrate target taxa and minimize PCR inhibitors and non-target DNA (Brannock and Halanych 2015; Nascimento et al. 2018; van der Loos and Nijland 2021). Comparing read abundances, 6.29% of the total reads in the ComDNA dataset were assigned to metazoans, representing 75.7% of OTUs, whereas only 1.27% of total reads and 25.9% of OTUs were detected in the SedDNA dataset. These findings demonstrate that DNA extracted directly from sediment recovers substantially fewer metazoan signals compared to extractions performed on pre-sorted organisms. This result is in agreement with previous studies (Fonseca et al. 2010; Sinniger et al. 2016; Derycke et al. 2021), which consistently demonstrate improved detection of benthic taxa when organisms are isolated from sediment before DNA extraction. Such preprocessing techniques, e.g., sieving and elutriation, not only reduce interference from non-target DNA but also concentrate benthic content, thereby improving DNA yield and quality (Creer et al. 2010; Hajibabaei et al. 2019; Castro et al. 2021; Gielings et al. 2021; He et al. 2021). ComDNA extraction strategies provide higher-resolution biodiversity assessments but demand significant resource investment and processing time due to the requirement for extensive sieving and decantation (Fonseca et al. 2010; Creer et al. 2010). Despite these logistical constraints, ComDNA metabarcoding remains crucial for accurate biodiversity monitoring and environmental assessments, particularly in ecologically sensitive areas such as the polymetallic nodule fields targeted for deep-sea mining (Wedding et al. 2015; Miller et al. 2018).

Sample volume also plays a critical role in shaping the observed diversity. As highlighted in earlier studies (Lejzerowicz et al. 2015; Aylagas et al. 2016; Pawlowski et al. 2022), determining the minimum volume of sediment necessary to accurately reflect the meiofaunal community is essential. In this study, SedDNA metabarcoding from small sample volumes recovered significantly fewer OTUs than ComDNA samples. To estimate the volume required to reach similar OTU richness, we applied a modeling approach using random subsampling of sample combinations (without replacement). The Michaelis–Menten model suggested a requirement of approximately 82.25 mL of sediment, while the linear model indicated a lower threshold of 46.5 mL. For instance, Nascimento et al. (2018) proposed that 14 g of sediment is sufficient for assessing beta diversity, and 22 g is needed to capture a representative portion of meiofaunal diversity in coastal environments. Similarly, Brandt et al. (2020) emphasized that processing more than 10 g of sediment is essential for reliably detecting deep-sea eukaryotes, reinforcing the conclusion that larger volumes are critical for robust community profiling. It should be noted that these estimates are reported in sediment volume (mL), whereas the cited studies report sediment mass (g). Although these units are not directly comparable without

knowing sediment density, the comparison still illustrates that our estimated sampling effort is larger than previous recommendations. Building on this, we explored how sequencing depth influences the sediment volume required for comparable OTU richness by rarefying the dataset at multiple depths, with a particular focus on the 100,000-reads dataset. Increasing sequencing depth reduced the estimated sediment volume necessary to achieve OTU richness comparable to ComDNA samples. For this dataset, the Michaelis–Menten model estimated a required volume of approximately 38.8 mL, while the linear model suggested a range between 27.0 mL (at maximum OTU richness) and 48.2 mL (at minimum OTU richness).

In addition to increasing sediment sample volume, the pretreatment of sediment samples, particularly homogenization, substantially improves biodiversity detection by ensuring that the DNA extract more accurately reflects the underlying community, despite small-scale spatial heterogeneity. This is especially critical in deep-sea environments, which exhibit high spatial heterogeneity even at small scales. The deep seafloor is known for its faunal patchiness, a feature that becomes even more pronounced in polymetallic nodule areas such as the CCZ (Tyler et al. 2016; Gollner et al. 2022). Montagna et al. (2017) showed that sediment cores from the same multicorer (MUC) deployment can display greater spatial variability than cores from spatially more distant deployments.

Meiofaunal communities exhibit pronounced microhabitat-scale heterogeneity (Brannock and Halanych 2015; Vanreusel et al. 2016; Nascimento et al. 2018), often clustering in regions rich in organic matter (Wang et al. 2019). As a result, homogenizing the entire sediment volume before SedDNA subsampling is strongly recommended to ensure more representative sampling and robust biodiversity estimates (Laroche et al. 2020; Hestetun et al. 2021; van der Loos and Nijland 2021). However, this approach comes with a clear trade-off: homogenization eliminates vertical stratification within the sediment, thereby obscuring depth-dependent patterns in community structure. This limitation can be avoided by homogenizing each sediment layer separately prior to subsampling if retaining vertical resolution is required.

Taxonomic composition and community representation

A large proportion of sequences, particularly in the SedDNA dataset, were assigned to non-metazoan taxa, primarily fungi and diverse microeukaryotes such as members of the SAR supergroup (Burki et al. 2007). While approximately 6.29% of high-quality sequences in the ComDNA dataset were assigned to metazoans, this proportion dropped to only 1.17% in the SedDNA dataset. The predominance of non-target amplification in sediment-derived DNA is known to impede meiofaunal detection (Brannock and Halanych 2015; Nascimento et al. 2018; Derycke et al. 2021).

Despite the observed differences between ComDNA and SedDNA, phylum-level patterns were broadly comparable between the two approaches. This is consistent with Brandt et al. (2021a), who reported similar high-level community patterns across extraction strategies when analyzing 18S rRNA metabarcoding data. In both datasets of our study, Annelida emerged as the most abundant phylum in terms of read counts, followed by Arthropoda in the SedDNA dataset and Nematoda in the ComDNA dataset, although relative abundances

differed. This general pattern aligns with studies using the same 18S V1–V2 marker (Liu and Zhang 2021) and several deep-sea meiofauna investigations (Bik et al. 2012; Guardiola et al. 2015), while contrasting with studies reporting nematodes as the dominant group (Guardiola et al. 2016; Sinniger et al. 2016; Hauquier et al. 2019; Macheriotou et al. 2020). Such discrepancies may arise not only from biological variation but also from methodological choices, including the selected gene region, bioinformatic workflow, and taxonomic reference database (Liu and Zhang 2021; Brandt et al. 2021b). The stronger dominance of annelids in SedDNA versus a more balanced composition in ComDNA also highlights biases inherent to extraction methods, and while SedDNA offers advantages such as faster processing and lower contamination risk (Atienza et al. 2020), it may under- or overrepresent taxa without careful standardization.

Importantly, differences in taxonomic assignment pipelines, particularly the reference database used, can substantially affect metabarcoding outputs and hinder comparisons across studies (Brandt et al. 2021b). In our study, taxonomy was assigned using BLAST against the NCBI database and a locally curated 18S V1–V2 barcode reference archive of the DZMB (Senckenberg am Meer, Wilhelmshaven). While assignment tools often yield broadly comparable results for 18S datasets, database completeness and taxonomic coverage generally exert a stronger influence on classification outcomes than the choice of algorithm itself (Brandt et al. 2021b). Additionally, the limited representation of deep-sea taxa in public databases further constrains low-level taxonomic resolution (Liu and Zhang 2021). Consequently, some differences between studies likely reflect variation in reference databases and bioinformatic processing rather than biological patterns alone. Our study incorporated taxa whose size or life history partly exceeded the classical meiofaunal definition, such as Chaetognatha, Bryozoa, Porifera, and some Annelida. These taxa were retained to maintain an unbiased, comprehensive comparison, acknowledging their relevance as partially benthic organisms and avoiding additional filtering biases. Furthermore, some macrofaunal phyla may be temporarily present within the meiofaunal size range, particularly as larval or juvenile stages (Giere 2009; Lins et al. 2021). Their detection in the ComDNA samples is therefore likely attributable to the inclusion of such early life stages, as well as eggs or tissue fragments (Fonseca et al. 2010; Fonseca et al. 2017), or due to the sieving of overlaying water above the sediment core during ComDNA sample processing, a step not included in SedDNA extraction workflows. Notably, four phyla were detected exclusively in the ComDNA dataset. For instance, Entoprocta, a group of small, sessile meiofaunal organisms often associated with polychaetes, sponges, and bryozoans (Nielsen 2002), may have been detected due to their free-swimming larval stages (Merkel et al. 2015). Similarly, OTUs assigned to Loricifera and Hemichordata, the latter specifically to the family Harrimanidae, which also includes larval forms, were found only in the ComDNA dataset (Burden-Jones 1952; Kristensen 2002; Borisanova 2019).

Considering the practical implications, our results clearly indicate that ComDNA extraction outperforms SedDNA methods for benthic biodiversity assessment, providing higher taxonomic resolution and more comprehensive detection of taxa. For studies aimed at establishing high-resolution biodiversity baselines or conducting environmental impact assessments, ComDNA

extraction is therefore the preferred approach, despite its greater effort and resource requirements. SedDNA methods, particularly with improved sample volumes, higher sequencing depths, and optimized processing protocols, are promising for broader monitoring applications, especially where rapid, large-scale surveys are needed.

Conclusion

This study demonstrates that the choice of DNA extraction strategy has a major influence on the recovery of metazoan diversity from deep-sea sediments. Isolating benthic taxa via centrifugation yielded higher richness and a more representative community composition than SedDNA extractions, underscoring their greater suitability for benthic community studies in deep-sea environments such as the CCZ. Our modeling approach further indicated that sediment volume is a critical factor: approximately 27–82 mL of sediment at a sufficient sequencing depth of at least 100,000 reads per sample would be required for SedDNA to recover richness levels comparable to ComDNA extractions. Given that high-capacity kits such as the Qiagen DNeasy PowerMax Soil Kit can process up to ~10 g of sediment per reaction, this translates to 3–9 biological replicates that are necessary to approach ComDNA-derived diversity.

Due to the pronounced spatial heterogeneity of deep-sea benthic habitats, particularly in polymetallic nodule regions such as the CCZ, homogenization of core sediments and standardized protocols for sediment processing may enhance comparability among SedDNA studies. However, these strategies remain to be explicitly tested. We therefore emphasize the need for further methodological research, including evaluation of sample volume, homogenization, and sequencing depth, and the inclusion of technical PCR replicates, to refine sediment-based eDNA protocols. At present, ComDNA organismal DNA extractions remain the more reliable strategy for capturing benthic community diversity, whereas optimized SedDNA approaches remain promising for scalable monitoring if their limitations are recognized and addressed.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Conceptualization: PMA, MM, VR, TS. Data curation: SK, LD. Funding acquisition: AV, TS, PMA, MM. Investigation: LD, VR. Methodology: TS, PMA, VR, MM, SK. Project administration: PMA, TS. Resources: PMA. Software: VR. Supervision: VR, SK, TS. Visualization: LD. Writing - original draft: LD, SK. Writing - review and editing: MM, AV, PMA, LD, TS, VR, SK.

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Data availability

The raw sequences are publicly available through the SRA Database of NCBI with project accession number PRJNA1308783. All scripts used in this study are publicly accessible on GitHub at https://github.com/dammlu/MIMeS/tree/main/Mangan18_eDNA_vs_Bulk. The data tables generated and analyzed during this study are publicly available on Zenodo (DOI: 10.5281/zenodo.16903244).

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Supplementary material 1

Additional information

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Data type: docx

Explanation note: **fig. S1.** nMDS of benthic metazoan community similarity across SedDNA layers. **fig. S2.** Estimated sediment sample size required to capture benthic metazoan OTU richness. **table S1.** Summary of pairwise PERMANOVA results comparing benthic metazoan community composition across individual SedDNA layers and between pooled SedDNA layers and ComDNA samples. Analyses used Bray–Curtis distance matrices with 9,999 permutations. **fig. S3.** Taxonomic composition of metazoan OTUs in SedDNA and ComDNA samples. **fig. S4.** Taxonomic composition of metazoan OTUs across separate SedDNA layers. **fig. S5.** Rarefied ASV richness in SedDNA and ComDNA samples.

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